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Carbazole Alkaloids from the Stems of *Clausena lansium*

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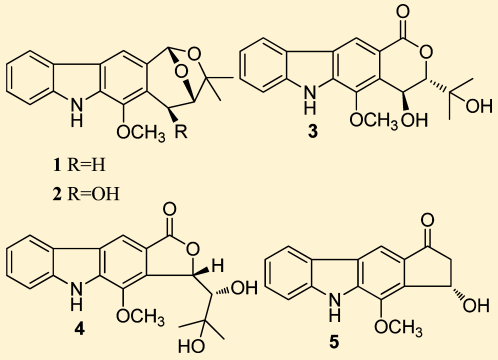
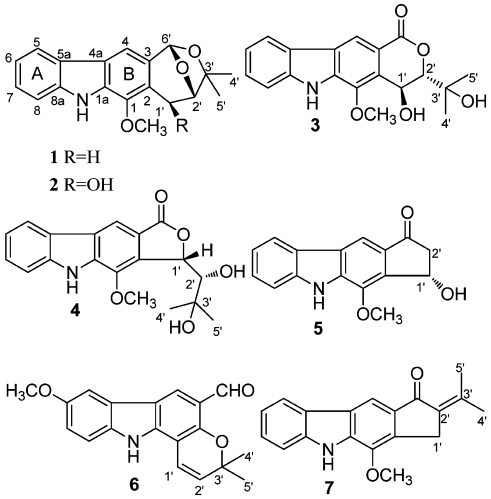
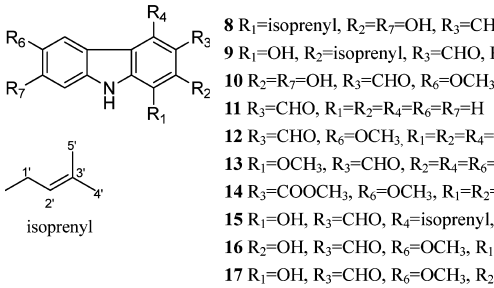
Supporting Information

ABSTRACT: Ten new carbazole alkaloids, claulansines A–J (**1**–**10**), and seven known analogues (**11**–**17**) were isolated from the stems of *Clausena lansium*. Their structures were established on the basis of extensive spectroscopic analyses, and their absolute configurations were determined by CD experiments and computational methods. Screening results indicated that compounds **1**, **6**, **8**–**10**, **13**, **14**, and **17** showed selective neuroprotective effects at the concentration of 10 μM.



Clausena lansium (Lour.) Skeels (Rutaceae), a fruit tree, is widely distributed in southern China. Several parts of this plant have been used as a folk medicine. The leaves have been used for the treatment of coughs, asthma, and gastro-intestinal diseases, and the seeds for acute and chronic gastro-intestinal inflammation and ulcers.[1] Previously, a megastigmane glucoside, four amide alkaloids, and seven flavonoid glycosides from the leaves of *C. lansium* were reported by our research group.[2,3] In a continuing search for bioactive metabolites from this plant, a 95% EtOH extract of the stems of *C. lansium* was investigated. Compounds isolated in the present study included 10 new carbazole alkaloids, claulansines A–J (**1**–**10**), and seven known carbazoles (**11**–**17**). We describe herein the isolation and structural elucidation of the new compounds and the determination of their absolute configurations through spectroscopic analysis, CD experiments, and computational methods. The neuroprotective activities of **1**–**17** are also evaluated.





8 R_1=isoprenyl, R_2=R_7=OH, R_3=CHO, R_6=OCH_3, R_4=H
9 R_1=OH, R_2=isoprenyl, R_3=CHO, R_4=R_6=R_7=H
10 R_2=R_7=OH, R_3=CHO, R_6=OCH_3, R_1=R_4=H
11 R_3=CHO, R_1=R_2=R_4=R_6=R_7=H
12 R_3=CHO, R_6=OCH_3, R_1=R_2=R_4=R_7=H
13 R_1=OCH_3, R_3=CHO, R_2=R_4=R_6=R_7=H
14 R_3=$COOCH_3$, R_6=OCH_3, R_1=R_2=R_4=R_7=H
15 R_1=OH, R_3=CHO, R_4=isoprenyl, R_2=R_6=R_7=H
16 R_2=OH, R_3=CHO, R_6=OCH_3, R_1=R_4=R_7=H
17 R_1=OH, R_3=CHO, R_6=OCH_3, R_2=R_4=R_7=H

RESULTS AND DISCUSSION

Claulansine A (**1**) was obtained as a white powder, $[\alpha]^{20}_{D}$ −63.7 (*c* 0.08, MeOH). The molecular formula, $C_{19}H_{19}NO_3$, was established by HRESIMS (332.1269 $[M + Na]^+$, calcd for 332.1257), implying 11 degrees of unsaturation. The IR spectrum displayed absorptions characteristic of amino (3394 cm^{-1}) and aromatic ring (1618, 1576, and 1505 cm^{-1}) groups, and the UV spectrum showed absorbances at λ_{max} 240, 248, and 294 nm. Four mutually coupling aromatic protons [δ_H 8.01 (1H, d, *J* = 7.5 Hz), 7.13 (1H, t, *J* = 7.5 Hz), 7.36 (1H, td, *J* = 7.5, 0.5 Hz), and 7.47 (1H, d, *J* = 7.5 Hz)]; a lone singlet at δ_H 7.60 (1H, s, H-4) in the aromatic proton region, and a methinedioxy proton at δ_H 6.09 (1H, s) were observed in the ^{1}H NMR spectrum of **1**. The ^{13}C NMR spectrum of **1** indicated the presence of 12 aromatic carbons, one methinedioxy, one oxygen-bearing quaternary carbon, one oxymethine, one methylene, one methoxyl, and two methyls. The above information

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coupled with biogenetic considerations and literature references[4,5] indicated the carbazole skeleton in **1**. In the HMBC experiment, correlation of H-4/δ_C 100.3 located the methinedioxy (C-6′) at C-3. A series of HMBC correlations from H-1′ to C-1, C-2, and C-3, from H-2′ to C-2 and C-6′, from H-6′ to C-2′ and C-3′, and from H-4′ and H-5′ to C-3′, taking the chemical shifts and the degree of unsaturation into consideration, revealed a modified isoprenyl unit from C-1′ to C-5′ attached to C-2 and that an oxygen atom at C-6′ was connected with C-3′ to form a seven-membered ring, while C-2′ was linked with another oxygen atom at C-6′. Additional HMBC correlation of δ_H 3.91/C-1 placed the OCH_3 at C-1. Thus, the planar structure of **1** was established.

The relative configuration of **1** indicated that there were only two possible structures, with the absolute configurations (2′*S*,6′*S*) or (2′*R*,6′*R*). The absolute configurations at C-2′ and C-6′ were established by theoretical calculations of its electronic circular dichroism (ECD) using the time-dependent density functional theory (TD-DFT) method.[6] Their optimized geometries were obtained, and then the ECD spectra were calculated at the B3LYP/6-31G(d) level with the TD-DFT/PCM model in methanol solution[7] (Supporting Information, S61, S62, and Table S1). As shown in Figure 1, the calculated ECD

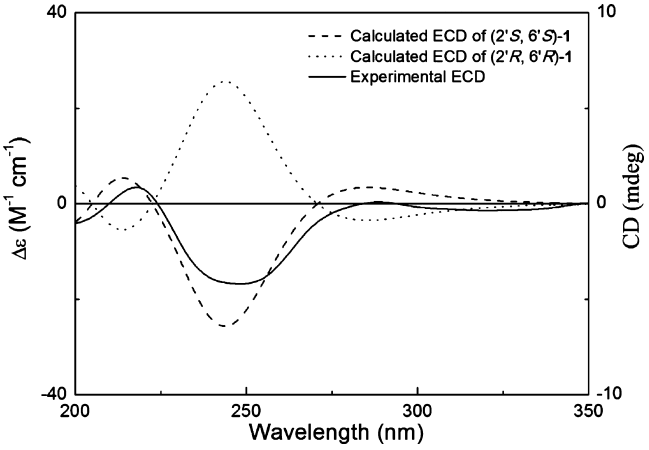

Figure 1. Calculated ECD spectra of the (2′*S*,6′*S*)- and (2′*R*,6′*R*)-isomers and the experimental ECD spectrum of **1**.

spectrum of the (2′*S*,6′*S*)-isomer exhibited a diagnostic negative Cotton effect at around 245 nm, corresponding to the experimental Cotton effect observed at 248 nm (Supporting Information, S5). Therefore, the absolute configurations at C-2′ and C-6′ were determined as 2′*S*,6′*S*. From these data, claulansine A was characterized as **1**.

Claulansine B (**2**) was isolated as a white powder, $[\alpha]^{20}_D$ −22.9 (*c* 0.09, MeOH). HRESIMS at *m*/*z* 326.1390 $[M + H]^+$ indicated that the molecular formula of **2** was $C_{19}H_{19}NO_4$. The UV, IR, and NMR data of **2** resembled those of **1**. The NMR analysis showed an additional OH at C-1′ (δ 61.2). That **2** was a 1′-oxygenated derivative of **1** was confirmed by the correlation of 1′-OH/C-1′ in the HMBC experiment.

A negative Cotton effect at 249 nm in the CD spectrum suggested that the absolute configurations at C-2′ and C-6′ of **2** were the same as those of **1**. On the basis of the bulkiness rule for secondary alcohols,[8] a positive Cotton effect at 344 nm in the $Rh_2(OCOCF_3)_4$-induced CD spectrum (Supporting Information, S11) indicated the 1′*S* configuration of **2**. Thus, claulansine B was characterized as **2**.

Claulansine C (**3**) gave a molecular formula of $C_{19}H_{19}NO_5$, as established by the HRESIMS ion at *m*/*z* 342.1343 $[M + H]^+$ (calcd for $C_{19}H_{20}NO_5$, 342.1336). The 1D NMR spectra of **3** displayed a carbazole skeleton having one ester carbonyl, two oxymethines, two methyl groups, one oxygen-bearing quaternary carbon, two OH groups, and one OCH_3. In the HMBC spectrum of **3**, correlations of H-1′/C-1, C-2, and C-3 and of H-2′/C-1′, C-2, and the ester carbonyl carbon suggested that C-2′ was connected to the C-3 ester carbonyl to form a six-membered lactone ring; correlations of δ_H 4.79, H-4′, and H-5′/ C-2′ and C-3′ indicated that a 2-hydroxyisopropyl group was linked with C-2′. Thus, the planar structure of **3** was established.

The relative configuration of **3** was deduced according to ROESY experiments. NOEs were observed between H-1′ and two methyls, while no NOE was observed between 1′-OH and two methyls, suggesting that the H-1′ and 2-hydroxyisopropyl group were on the same side of the six-membered lactone ring. There were only two possible structures considered for **3**, with the absolute configurations (1′*S*,2′*S*) and (1′*R*,2′*R*). A positive Cotton effect at 350 nm in the $Rh_2(OCOCF_3)_4$-induced CD spectrum[8] (Supporting Information, S18) indicated the 1′*S*,2′*S* configuration of **3**.

Claulansine D (**4**) was assigned the same molecular formula, $C_{19}H_{19}NO_5$, as **3** by HRESIMS. The 1D NMR spectra were similar to those of **3**, especially in the lower field, suggesting that **4** had the same substitutions on the carbazole skeleton as **3**. In the HMBC spectrum of **4**, correlations of the H-1′/ester carbonyl carbon, C-1, C-2, and C-3 suggested that C-1′ was connected with the C-3 ester carbonyl to form a five-membered lactone ring. HMBC correlations of H-2′/C-2, C-1′, C-3′, C-4′, and C-5′ and of δ_H 4.78, H-4′, and H-5′/C-3′ allowed linkage of the side chain as shown in **4**.

The absolute configuration at C-1′ of **4** was presumed to be 1′*S* by the hypothetical biogenetic pathway (Supporting Information, S65). Compounds **3** and **4** could be derived from indizoline.[4] Oxidation of the isoprenyl and aldehyde of indizoline followed by perhydroxylation and esterification produced **3** and **4**. Thus, the absolute configuration at C-1′ of **4** should be the same as C-1′ of **3**.

The absolute configuration of the 2′,3′-diol moiety was determined using induced CD spectra by Snatzke's method.[10,11] A positive Cotton effect at 303 nm in the induced CD spectrum (Supporting Information, S24) indicated the 2′*S* configuration for **4** by means of the empirical helicity rule. Hence, claulansine D was characterized as **4**.

The molecular formula of claulansine E (**5**) was determined to be $C_{16}H_{13}NO_3$ (HRESIMS). Comparion of the 1D NMR spectra with those of **3** and **4** suggested that **5** was also a 1-oxygenated 2,3-substituted carbazole derivative with no substituent on the A ring. The HMBC spectrum of **5** displayed correlations of H-2′/C-2, C-3, C-1′, and the carbonyl carbon, suggesting that C-1′, C-2′, C-2, C-3, and the C-3 carbonyl carbon established a five-membered ring. In the $Rh_2(OCOCF_3)_4$-induced CD spectrum[8] (Supporting Information, S30), a positive Cotton effect at 354 nm indicated the 1′*S* configuration for **5**.

The spectroscopic data of claulansine F (**6**) indicated that it was also a carbazole alkaloid. The molecular formula, $C_{19}H_{17}NO_3$, was established by HRESIMS. In the ^1H NMR spectrum, a ABX system [δ_H 7.35 (1H, d, *J* = 8.5 Hz), 6.98 (1H, dd, *J* = 8.5, 2.5 Hz), and 7.74 (1H, d, *J* = 2.5 Hz)] suggested the presence of one substituent on the A ring attached to either C-6 or C-7; a downfield-shifted aromatic proton signal at δ_H 8.35

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was assigned to H-4; two doublets at δ_H 6.93 (1H, d, J = 10.0 Hz) and 5.91 (1H, d, J = 10.0 Hz) were the signals of the *cis* olefinic protons. In the HMBC experiment, correlation of H-4/δ_C 187.8 located the aldehyde group at C-3; correlations of H-1′/C-1a, C-1, C-2, and C-3′ and of H-4′ and H-5′/C-2′ and C-3′, as well as their chemical shifts and the degree of unsaturation, suggested that an isoprenyl unit was attached to C-1, and an oxygen atom at C-2 was connected with C-3′ to form a six-membered ring. In the ROESY spectrum, correlations of δ_H 11.59/δ_H 6.93 and 7.35 and δ_H 6.98/δ_H 7.35 and 3.82 verified that the OCH_3 group was attached to C-6. Therefore, claulansine F was characterized as **6**.

The elemental composition of claulansine G (**7**) was established as $C_{19}H_{17}NO_2$ by HRESIMS. On the basis of analysis of the IR, UV, and 1D NMR spectra, **7** had the same substitutions on the carbazole skeleton as **3**, **4**, and **5**. In the HMBC spectrum of **7**, correlations of H-1′/C-1, C-2, C-2′, and the carbonyl carbon and of H-4′ and H-5′/C-2′ and C-3′ suggested that an isoprenyl unit was attached to C-2 and that C-2′ was connected with the C-3 carbonyl group to establish a five-membered ring. In the NOESY experiment, correlations of H-1′/H-4′ and 1-OCH_3 and of H-4′/H-1′ and H-5′ confirmed the structure as shown.

Claulansine H (**8**) was determined to be $C_{19}H_{19}NO_4$ (by HRESIMS), and the ^{1}H and ^{13}C NMR data indicated that **8** was a pentasubstituted carbazole. In the HMBC spectrum, correlations of H-1′/C-1, C-2, and C-1a suggested that the isoprenyl group was attached to C-1; correlations of δ_H 11.48/C-1, C-2, and C-3 placed the OH at C-2; correlations of δ_H 9.87/C-2, C-3, and C-4 located the aldehyde at C-3. The NOESY correlation of δ_H 3.85 with H-5 (δ 7.60) confirmed that the OCH_3 was attached to C-6. This is the first naturally occurring pentasubstituted carbazole.

Claulansine I (**9**) gave a molecular formula of $C_{18}H_{17}NO_2$, and analysis of 1D NMR data revealed that **9** was a trisubstituted carbazole. The HMBC correlations of H-1′/C-1, C-2, and C-3 and of H-4/δ_C 192.3 located the isoprenyl group at C-2 and the aldehyde at C-3, respectively. Hence, claulansine I was characterized as **9**.

The spectroscopic data of claulansine J (**10**) indicated that it was a tetrasubstituted carbazole. In the HMBC spectrum, correlations of the aldehyde (δ 10.07)/C-2, C-3, and C-4 indicated it was linked with C-3. In the NOE difference spectrum, irradiation of the OCH_3 at δ_H 3.85 gave an enhancement of δ_H 7.61 (H-5), while δ_H 6.85 and 6.77 were enhanced when the amino group (δ 11.16) was irradiated, suggesting that the OCH_3 was attached to C-6 and that OH groups were attached to C-2 and C-7, respectively.

Seven known carbazole alkaloids, 3-formylcarbazole (**11**),[9] 3-formyl-6-methoxy carbazole (**12**),[9] murrayanine (**13**),[9] methyl 6-methoxycarbazole-3-carboxylate (**14**),[9] clausine-D (**15**),[12] glycozolidal (**16**),[13] and clausine-I (**17**),[14] were also identified on the basis of their spectroscopic profiles (NMR, UV, and MS) and comparison to published data.

Compounds **1**−**17** were evaluated for their neuroprotective effect on neuron-like PC12 cells induced by serum withdrawal, Aβ_{25-35}, and sodium nitroprusside (SNP)[15] in vitro using the MTT method. In the aforementioned two models, the neuron growth factor (NGF) was used as a positive control, while the edaravone, as a reactive oxygen species scavenger, was taken as an antioxidant positive control in the SNP-induced model. At 10 μM, **1**, **8**−**10**, and **13** increased the cell survival rate of the Aβ_{25-35}-treated group, while **1**, **6**, **10**, **13**, **14**, and **17** blocked the toxicity induced by SNP; all the compounds failed to protect cells from serum withdrawal. The other compounds were inactive (Supporting Information, TableS2).

EXPERIMENT SECTION

General Experimental Procedures. Optical rotations were measured on a P2000 automatic digital polarimeter. UV spectra were taken with a Hitachi UV-240 spectrophotometer. CD spectra were measured on a JASCO *J*-815 spectropolarimeter. IR spectra were recorded on a Nicolet 5700 FT-IR spectrometer. NMR measurements were performed on INOVA-500, Mercury-plus-400, and Bruker AV500-III spectrometers in DMSO-d_6. HRESIMS were obtained using an Agilent 1100 series LC/MSD Trap SL mass spectrometer. Preparative HPLC was conducted using a Shimadazu LC-6AD instrument with a SPD-20A detector and a YMC-Pack ODS-A column (250 × 20 mm, 5 μm). Column chromatography (CC) was performed with silica gel (200−300 mesh, Qingdao Marine Chemical Inc., Qingdao, People's Republic of China) and ODS (50 μm, YMC, Japan). TLC was carried out on glass precoated silica gel GF_{254} plates. Spots were visualized under UV light or by spraying with 10% sulfuric acid in EtOH followed by heating.

Plant Material. The stems of *C. lansium* were collected in Liuzhou, Guangxi, China, in December 2008 and identified by Engineer Guangri Long, Forestry of Liuzhou. A voucher specimen has been deposited at the Herbarium of Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College (ID-S-2320).

Extraction and Isolation. Air-dried, powdered stems of *C. lansium* (6.4 kg) were macerated for 3 h with 20 L of 95% EtOH(aq) and refluxed for 4 h (20 L × 2). The filtrate was evaporated under reduced pressure to yield a dark brown residue (640 g). The residue was suspended in water (2000 mL) and then partitioned with $CHCl_3$ (3 × 2000 mL), EtOAc (3 × 2000 mL), and *n*-BuOH (3 × 2000 mL), successively. After removing solvent, the $CHCl_3$-soluble portion (195 g) was fractionated via silica gel CC eluting with petroleum ether−acetone (3:1) to afford 10 fractions, A_1−A_{10}, on the basis of TLC analysis. Fraction A_2 (7.301 g) was chromatographed over silica gel (200−300 mesh) eluted with petroleum ether−acetone gradients (6:1, 4:1) to give five subfractions, A_2a−A_2e. Separation of fraction A_2d (3.12 g) by MPLC (50−100% MeOH−H_2O) and preparative HPLC (detection at 210 nm, 7 mL/min), successively, yielded **7** (10 mg), **8** (7 mg), **9** (5 mg), and **15** (14 mg). Fraction A_3 (6.483 g) was chromatographed over silica gel (200−300 mesh) eluted with petroleum ether−acetone gradients (5:1, 4:1, 3:1) to give four subfractions, A_3a−A_3d. The carbazole-containing fraction A_3b (2.12 g) was purified by MPLC (50−100% MeOH−H_2O) followed by preparative HPLC (detection at 210 nm, 7 mL/min) to yield **11** (11 mg), **12** (9 mg), **13** (14 mg), **14** (6 mg), **1** (20 mg), **16** (13 mg), and **6** (15 mg). Fraction A_9 (29.83 g) was chromatographed over silica gel (200−300 mesh) eluted with $CHCl_3$−MeOH gradients (20:1, 8:1, 4:1) to give nine subfractions, A_9a−A_9i. Fraction A_9c (5.13 g) was subjected to MPLC (25−60% MeOH−H_2O) successively using preparative HPLC (detection at 210 nm, 7 mL/min) to yield **10** (13 mg), **3** (3 mg), **4** (2 mg), **5** (1 mg), **2** (5 mg), and **17** (3 mg).

Claulansine A (1): white powder; $[\alpha]^{20}_D$ −63.7 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 240 (4.14), 248 (4.07), 294 (3.76) nm; IR ν_{max} 3394, 2978, 2843, 1703, 1618, 1576, 1505, 1460, 1359, 1316, 1071, 974, 757 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 125 MHz), see Tables 1 and 2; CD (MeOH) 218 ($\Delta\varepsilon$ +0.86), 248 ($\Delta\varepsilon$ −4.20), 289 ($\Delta\varepsilon$ +0.09), 321 ($\Delta\varepsilon$ −0.35) nm; HRESIMS *m*/*z* 332.1269 $[M + Na]^+$ (calcd for $C_{19}H_{19}NO_3Na$, 332.1257).

Claulansine B (2): white powder; $[\alpha]^{20}_D$ −22.9 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε) 242 (4.13), 250 (4.06), 295 (3.74) nm; IR ν_{max} 3387, 2974, 1715, 1620, 1579, 1505, 1460, 1427, 1361, 1082, 931, 773 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 125 MHz), see Tables 1 and 2; CD (MeOH) 224 ($\Delta\varepsilon$ +1.49), 249 ($\Delta\varepsilon$ −3.79), 282 ($\Delta\varepsilon$ +0.52), 330 ($\Delta\varepsilon$ −0.35) nm; $Rh_2(OCOCF_3)_4$-induced CD (CH_2Cl_2) 344 ($\Delta\varepsilon$ +0.23) nm; HRESIMS *m*/*z* 326.1390 $[M + H]^+$ (calcd for $C_{19}H_{19}NO_4$, 326.1387).

Claulansine C (3): white powder; $[\alpha]^{20}_D$ +4.5 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε) 236 (3.88), 269 (4.10) nm; IR ν_{max} 3347, 2974,

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Table 1. ^{1}H NMR Data of Compounds 1−10[a]

position	1	2	3	4	5	6	7	8	9	10
1										6.78 s
4	7.60 s	7.62 s	8.55 s	8.37 s	8.19 s	8.35 s	8.23 s	8.17 s	8.21 s	8.26 s
5	8.01 d (7.5)	8.04 d (8.0)	8.23 d (7.5)	8.27 d (7.5)	8.23 d (7.5)	7.74 d (2.5)	8.21 d (7.5)	7.59 s	8.13 d (8.0)	7.61 s
6	7.13 t (7.5)	7.14 t (7.5)	7.22 t (8.0)	7.22 t (7.5)	7.20 t (7.0)		7.19 td (8.0, 0.5)		7.20 t (7.5)	
7	7.36 td (7.5, 0.5)	7.38 t (7.5)	7.46 t (7.5)	7.46 t (7.5)	7.44 t (7.5)	6.98 dd (8.5, 2.5)	7.42 td (8.0, 1.0)		7.40 t (7.5)	
8	7.47 d (7.5)	7.50 d (8.0)	7.56 d (8.0)	7.55 d (8.0)	7.53 d (8.0)	7.35 d (8.5)	7.52 d (8.0)	6.93 s	7.56 d (8.0)	6.85 s
1′	3.21 dd (18.0, 5.5), 3.01 d (17.5)	4.77 d (7.5)	5.38 d (6.0)	6.16 s	5.60 m	6.93 d (10.0)	3.85 s	3.50 d (7.0)	3.93 d (7.0)	
2′	4.49 d (5.0)	4.30 s	4.44 s	3.89 d (7.5)	3.11 dd (18.5, 6.5), 2.49[b]	5.91 d (10.0)		5.28 t (7.0)	5.16 t (7.0)	
4′	1.26 s	1.27 s	0.74 s	1.22 s		1.49 s	2.01 s	1.63 s	1.62 s	
5′	1.15 s	1.04 s	1.14 s	1.26 s		1.49 s	2.39 s	1.78 s	1.77 s	
1-OH									9.35 brs	
1-OCH_3	3.91 s	4.00 s	4.04 s	4.06 s	4.18 s		4.08 s			
2-OH								11.48 s		10.87 brs
3-CHO						10.35 s		9.87 s	10.14 s	10.07 s
3-O-CH-O-(6′)	6.09 s	6.11 s								
6-OCH_3						3.82 s		3.84 s		3.84 s
7-OH								9.18 s		9.14 brs
NH	11.29 brs	11.38 brs	11.84 brs	11.80 brs	11.75 brs	11.59 brs	11.66 brs	11.18 brs	11.37 brs	11.16 brs
1′-OH		5.34 d (7.5)	5.71 d (6.0)		5.64 d (7.0)					
2′-OH				4.80 d (7.5)						
3′-OH			4.79 s	4.79 s						

[a] ^{1}H NMR data (δ) were measured in DMSO-d_6 at 500 MHz for **1**−**9** and at 400 MHz for **10**. [b] Signal overlapped by solvent peaks.

Table 2. ^{13}C NMR Data of Compounds 1−10[a]

position	1	2	3	4	5	6	7	8	9	10
1	142.4	144.3	141.1	138.6	141.9	103.9	140.6	108.7	139.9	96.0
1a	132.3	132.6	135.8	136.3	137.3	141.0	136.7	144.4	134.5	145.6
2	120.1	123.2	129.2	135.1	140.4	153.6	133.5	155.6	126.1	158.8
3	130.1	129.1	116.3	118.8	129.6	117.1	133.2	114.3	126.9	115.2
4	111.7	111.2	118.0	112.7	110.0	119.5	111.4	124.1	118.7	123.0
4a	122.8	122.7	124.1	122.6	125.9	117.7	125.0	117.7	121.4	117.8
5	119.8	120.1	120.8	121.0	121.0	103.5	120.8	103.4	120.4	103.6
5a	122.1	123.6	122.7	126.3	122.9	123.8	123.1	114.3	123.1	114.1
6	118.7	118.8	119.8	119.6	119.5	154.0	119.5	143.6	119.6	143.5
7	125.3	125.8	126.6	126.7	126.6	114.5	126.4	146.6	126.0	146.5
8	111.2	111.4	111.6	111.7	111.7	111.7	111.6	98.2	111.7	98.0
8a	139.8	140.1	140.5	140.8	140.9	135.2	140.7	135.8	139.9	135.6
1′	25.5	61.2	57.0	77.6	65.2	116.7	29.1	22.6	23.4	
2′	79.2	86.0	90.4	75.8	47.9	129.7	130.7	121.7	124.2	
3′	79.8	76.9	70.3	71.6		77.0	147.2	131.6	130.2	
4′	29.4	29.7	25.6	28.5		27.2	24.1	25.4	25.5	
5′	23.6	23.2	28.0	24.7		27.2	19.8	17.9	18.0	
1-OCH_3	59.7	61.0	61.7	60.3	60.0		59.9			
3-CHO						187.8		195.8	192.3	192.5
3-COO-			164.0	170.8						
3-O-CH-O-(6′)	100.3	100.3								
3-CO−					202.7		192.3			
6-OCH_3						55.5		56.2		56.3

[a] ^{13}C NMR data (δ) were measured in DMSO-d_6 at 125 MHz for **1**−**9** and at 100 MHz for **10**.

2906, 1697, 1612, 1585, 1460, 1367, 1251, 1037, 748 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 125 MHz), see Tables 1 and 2; CD (MeOH) 228 ($\Delta\varepsilon$ +0.88), 276 ($\Delta\varepsilon$ −0.29), 294 ($\Delta\varepsilon$ +0.33) nm; $Rh_2(OCOCF_3)_4$-induced CD(CH_2Cl_2) 350

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($\Delta\varepsilon$ +0.35) nm; HRESIMS m/z 342.1343 $[M + H]^+$ (calcd for $C_{19}H_{20}NO_5$, 342.1336).

*Claulansine D (**4**):* white powder; $[\alpha]^{20}_D$ −81.2 (*c* 0.09, MeOH); UV (MeOH) λ_{max} (log ε) 229 (3.84), 278 (4.05) nm; IR ν_{max} 3382, 2988, 2945, 1736, 1617, 1591, 1494, 1395, 1249, 1101, 759 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 125 MHz), see Tables 1 and 2; CD (MeOH) 228 ($\Delta\varepsilon$ +0.30), 242 ($\Delta\varepsilon$ −0.51), 265 ($\Delta\varepsilon$ +1.48) nm; $Mo_2(OAc)_4$-induced CD (DMSO) 303 ($\Delta\varepsilon$ +0.28) nm; HRESIMS m/z 364.1163 $[M + Na]^+$ (calcd for $C_{19}H_{19}NO_5Na$, 364.1155).

*Claulansine E (**5**):* white powder; $[\alpha]^{20}_D$ +3.6 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 228 (3.70), 289 (4.09) nm; IR ν_{max} 3475, 3240, 2965, 1687, 1603, 1517, 1460, 1345, 1246, 746 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 125 MHz), see Tables 1 and 2; CD (MeOH) 241 ($\Delta\varepsilon$ +0.48), 285 ($\Delta\varepsilon$ +0.22), 335 ($\Delta\varepsilon$ +0.15) nm; $Rh_2(OCOCF_3)_4$-induced CD(CH_2Cl_2) 354 ($\Delta\varepsilon$ +0.14) nm; HRESIMS m/z 290.0788 $[M + Na]^+$ (calcd for $C_{16}H_{13}NO_3Na$, 290.0789).

*Claulansine F (**6**):* yellow powder; UV (MeOH) λ_{max} (log ε) 227 (4.07), 311 (4.09) nm; IR ν_{max} 3407, 3163, 3074, 2985, 1641, 1588, 1475, 1302, 1257, 1218, 1149, 1034 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 125 MHz), see Tables 1 and 2; HRESIMS m/z 330.1119 $[M + Na]^+$ (calcd for $C_{19}H_{17}NO_3Na$, 330.1101).

*Claulansine G (**7**):* light yellow powder; UV (MeOH) λ_{max} (log ε) 231 (4.07), 305 (4.27) nm; IR ν_{max} 3356, 3321, 2940, 2848, 1674, 1603, 1491, 1458, 1347, 1292, 1247, 1082, 838, 742 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 125 MHz), see Tables 1 and 2; HRESIMS m/z 314.1154 $[M + Na]^+$ (calcd for $C_{19}H_{17}NO_2Na$, 314.1151).

*Claulansine H (**8**):* yellow powder; UV (MeOH) λ_{max} (log ε) 206 (4.08), 311 (4.27) nm; IR ν_{max} 3604, 3411, 2963, 1642, 1593, 1440, 1360, 1233, 951, 768 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 125 MHz), see Tables 1 and 2; HRESIMS m/z 348.1202 $[M + Na]^+$ (calcd for $C_{19}H_{19}NO_4Na$, 348.1206).

*Claulansine I (**9**):* light yellow powder; UV (MeOH) λ_{max} (log ε) 202 (4.00), 275 (4.17) nm; IR ν_{max} 3334, 2935, 2866, 1655, 1597, 1311, 1241, 1200, 736 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 125 MHz), see Tables 1 and 2; HRESIMS m/z 280.1332 $[M + H]^+$ (calcd for $C_{18}H_{18}NO_2$, 280.1338).

*Claulansine J (**10**):* brown powder; UV (MeOH) λ_{max} (log ε) 204 (3.85), 224 (3.83) 310 (4.37) nm; IR ν_{max} 3480, 3369, 2946, 2843, 1700, 1612, 1549, 1487, 1359, 1274, 1216, 1157, 740 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) and ^{13}C NMR (DMSO-d_6, 100 MHz), see Tables 1 and 2; HRESIMS m/z 280.0580 $[M + Na]^+$ (calcd for $C_{14}H_{11}NO_4Na$, 280.0580).

Determination of Absolute Configuration of the Secondary Alcohol Units in 2, 3, and 5 (ref 8). Following the reported procedure, a 1:2 mixture of secondary alcohol−$Rh_2(OCOCF_3)_4$ for **2**, **3**, and **5** was subjected to CD measurements at a concentration of 0.1 mg/mL in anhydrous CH_2Cl_2. The first CD spectrum was recorded immediately after mixing, and its time evolution was monitored until stationary (about 10 min after mixing). The inherent CD was subtracted. The observed sign of the band at around 350 nm in the induced CD spectrum was correlated to the absolute configuration of the secondary alcohol.

Determination of Absolute Configuration of the 2′,3′-Diol Unit in 4 by Snatzke's Method (refs 10, 11). According to the published procedure, a 1:1.2 mixture of diol/$Mo_2(OAc)_4$ for **4** was subjected to CD measurements at a concentration of 0.1 mg/mL in anhydrous DMSO. The first CD spectrum was recorded immediately after mixing, and its time evolution was monitored until stationary (about 10 min after mixing). The inherent CD was subtracted. The observed sign of the diagnostic band at around 300 nm in the induced CD spectrum was correlated to the absolute configuration of the 2′,3′-diol unit.

Neuroprotection Bioassays. Pheochromocytoma (PC12) cells were incubated in DMEM supplied with 5% fetal bovine serum and 5% equine serum as basic medium. PC12 cells in logarithmic phase were cultured at a density of 5000 cells per well in a 96-well microtiter plate. After 24 h incubation, the medium of the model group was changed to DMEM or basic medium with 15 μM Aβ_{25-35} for 48 h or basic medium with 350 μM SNP for 24 h. Test compounds dissolved in dimethyl sulfoxide (DMSO) were added to each well for >1000-fold dilution in the model medium at the same time. Each sample was tested in triplicate. After the incubation at 37 °C in 5% CO_2 for 24 h, 10 μL of MTT (5 mg/mL) was added to each well and incubated for another 4 h; then liquid in the wells was removed. DMSO (100 μL) was added to each well. The absorbance was recorded on a microplate reader (Bio-Rad model 550) at a wavelength of 570 nm. Analysis of variance (ANOVA) followed by Newman−Keuls post hoc test were performed to assess the differences between the relevant control and each experimental group. *p*-Values of <0.05, < 0.01, and <0.001 were regarded as statistically significant. Data were expressed as mean ± SEM as indicated.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Copies of spectra of compounds **1**−**10**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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